activity and matrix environment are interdependent.[16] Therefore, *COL4A6* depletion in ovarian extracellular matrix may alter normal steroidogenesis even in the ovary and have been possibly the cause of POF, especially in case 1. So far, there have been at least eight POF genes registered in OMIM: *FMR1* at Xq27.3 (POF1, OMIM no. 311360), *DIAPH2* at Xq22 (POF2A, OMIM no. 300511), *POF1B* at Xq21 (POF2B, OMIM no. 300604), *FOXL2* at 3q23 (POF3, OMIM no. 608996), *BMP15* at Xp11.2 (POF4, OMIM no. 300510), *NOBOX* at 7q35 (POF5, OMIM no. 611548), *FIGLA* at 2p12 (POF6, OMIM no. 612310) and *NR5A1* at 9q33 (POF7, OMIM no. 312964). Furthermore, *XPNPEP2* at Xq25,[17] *DACH2* at Xq21.2[18] and *CHM* (Xq21.2)[19] have also been described as being disrupted by translocations. *COL4A6* may possibly be an additional X-linked gene related to POF.

Two autosomal genes were disrupted: a gene encoding *IGFBP7* at 4q12 and *C14orf159* on 14q32.12. *IGFBP7* (also known as *IGFBP-rP1* or *MAC25*) is a secreted 31-kDa protein, belonging to the IGFBP superfamily. *IGFBP7* is involved in proliferation, senescence and apoptosis. Recently, it is reported that *IGFBP7* loss has a functional role in thyroid carcinogenesis.[20] *C14orf159* is a hypothetical protein with unknown function. Both disrupted genes were relatively expressed in ovary based on the GeneCards database (http://www.genecards.org/). We could not find any sequence aberrations in either gene among other POF patients. Further analysis might be necessary in relation to POF.

According to the precise breakpoint locations in all the cases reported here, *COL4A5* and *IRS4* (case 2), *NXF2* (case 3) and *KLHL13* (case 4) were localized near to breakpoints (within less than a 100-kb distance). All the adjacent genes except for *KLHL13* are shown to be expressed in human ovary in the GeneCards database. Interestingly, it was suggested that IRS4 protein expression was decreased in theca cells of polycystic ovary syndrome[21] and IGFBP7 protein suppressed estrogen production in granulose cells.[22] Reduced expression of these genes owing to the position effects by translocations could affect to normal ovarian function.

On the basis of the breakpoint sequences, two translocations (in cases 2 and 4) had microhomology (defined as the presence of the same short sequence of bases) of a few nucleotides and the other two (in cases 1 and 3) showed insertion of 3–8 nucleotides of unknown origin, suggesting that non-homologous end-joining is related to the formation of all the translocations in our patients.[23]

In conclusion, we could determine four t(X;autosome) breakpoints at the nucleotide level. We found that only one X-linked gene, *COL4A6*, was disrupted, resulting in functionally null status. All the four translocations are formed by non-homologous end-joining.

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1 Coulam, C. B., Adamson, S. C. & Annegers, J. F. Incidence of premature ovarian failure. *Obstet. Gynecol.* **67**, 604–606 (1986).
2 Therman, E., Laxova, R. & Susman, B. The critical region on the human Xq. *Hum. Genet.* **85**, 455–461 (1990).
3 Schlessinger, D., Herrera, L., Crisponi, L., Mumm, S., Percesepe, A., Pellegrini, M. *et al.* Genes and translocations involved in POF. *Am. J. Med. Genet.* **111**, 328–333 (2002).
4 Rizzolio, F., Bione, S., Sala, C., Goegan, M., Gentile, M., Gregato, G. *et al.* Chromosomal rearrangements in Xq and premature ovarian failure: mapping of 25 new cases and review of the literature. *Hum. Reprod.* **21**, 1477–1483 (2006).
5 Sala, C., Arrigo, G., Torri, G., Martinazzi, F., Riva, P., Larizza, L. *et al.* Eleven X chromosome breakpoints associated with premature ovarian failure (POF) map to a 15-Mb YAC contig spanning Xq21. *Genomics* **40**, 123–131 (1997).
6 Rizzolio, F., Sala, C., Alboresi, S., Bione, S., Gilli, S., Goegan, M. *et al.* Epigenetic control of the critical region for premature ovarian failure on autosomal genes translocated to the X chromosome: a hypothesis. *Hum. Genet.* **121**, 441–450 (2007).
7 Bano, G., Mansour, S. & Nussey, S. The association of primary hyperparathyroidism and primary ovarian failure: a *de novo* t(X; 2) (q22p13) reciprocal translocation. *Eur. J. Endocrinol.* **158**, 261–263 (2008).
8 Allen, R. C., Zoghbi, H. Y., Moseley, A. B., Rosenblatt, H. M. & Belmont, J. W. Methylation of *Hpa*II and *Hha*I sites near the polymorphic CAG repeat in the human androgen-receptor gene correlates with X chromosome inactivation. *Am J Hum. Genet.* **51**, 1229–1239 (1992).
9 Carrel, L. & Willard, H. F. An assay for X inactivation based on differential methylation at the fragile X locus, FMR1. *Am. J. Med. Genet.* **64**, 27–30 (1996).
10 Kubota, T., Nonoyama, S., Tonoki, H., Masuno, M., Imaizumi, K., Kojima, M. *et al.* A new assay for the analysis of X-chromosome inactivation based on methylation-specific PCR. *Hum. Genet.* **104**, 49–55 (1999).
11 Khoshnoodi, J., Pedchenko, V. & Hudson, B. G. Mammalian collagen IV. *Microsc. Res. Technol.* **71**, 357–370 (2008).
12 Borza, D. B., Bondar, O., Ninomiya, Y., Sado, Y., Naito, I., Todd, P. *et al.* The NC1 domain of collagen IV encodes a novel network composed of the alpha 1, alpha 2, alpha 5, and alpha 6 chains in smooth muscle basement membranes. *J. Biol. Chem.* **276**, 28532–28540 (2001).
13 Zhou, J., Ding, M., Zhao, Z. & Reeders, S. T. Complete primary structure of the sixth chain of human basement membrane collagen, alpha 6(IV). Isolation of the cDNAs for alpha 6(IV) and comparison with five other type IV collagen chains. *J. Biol. Chem.* **269**, 13193–13199 (1994).
14 Sugimoto, K., Yanagida, H., Yagi, K., Kuwajima, H., Okada, M. & Takemura, T. A Japanese family with Alport syndrome associated with esophageal leiomyomatosis: genetic analysis of COL4A5 to COL4A6 and immunostaining for type IV collagen subtypes. *Clin. Nephrol.* **64**, 144–150 (2005).
15 Hertz, J. M., Juncker, I. & Marcussen, N. MLPA and cDNA analysis improves COL4A5 mutation detection in X-linked Alport syndrome. *Clin. Genet.* **74**, 522–530 (2008).
16 Mazaud Guittot, S., Verot, A., Odet, F., Chauvin, M. A. & le Magueresse-Battistoni, B. A comprehensive survey of the laminins and collagens type IV expressed in mouse Leydig cells and their regulation by LH/hCG. *Reproduction* **135**, 479–488 (2008).
17 Prueitt, R. L., Ross, J. L. & Zinn, A. R. Physical mapping of nine Xq translocation breakpoints and identification of XPNPEP2 as a premature ovarian failure candidate gene. *Cytogenet. Cell Genet.* **89**, 44–50 (2000).
18 Prueitt, R. L., Chen, H., Barnes, R. I. & Zinn, A. R. Most X;autosome translocations associated with premature ovarian failure do not interrupt X-linked genes. *Cytogenet. Genome Res.* **97**, 32–38 (2002).
19 Lorda-Sanchez, I. J., Ibanez, A. J., Sanz, R. J., Trujillo, M. J., Anabitarte, M. E., Querejeta, M. E. *et al.* Choroideremia, sensorineural deafness, and primary ovarian failure in a woman with a balanced X-4 translocation. *Ophthalmic Genet.* **21**, 185–189 (2000).
20 Vizioli, M. G., Sensi, M., Miranda, C., Cleris, L., Formelli, F., Anania, M. C. *et al.* IGFBP7: an oncosuppressor gene in thyroid carcinogenesis. *Oncogene* **29**, 3835–3844 (2010).
21 Yen, H. W., Jakimiuk, A. J., Munir, I. & Magoffin, D. A. Selective alterations in insulin receptor substrates-1, -2 and -4 in theca but not granulosa cells from polycystic ovaries. *Mol. Hum. Reprod.* **10**, 473–479 (2004).
22 Tamura, K., Matsushita, M., Endo, A., Kutsukake, M. & Kogo, H. Effect of insulin-like growth factor-binding protein 7 on steroidogenesis in granulosa cells derived from equine chorionic gonadotropin-primed immature rat ovaries. *Biol. Reprod.* **77**, 485–491 (2007).
23 Gu, W., Zhang, F. & Lupski, J. R. Mechanisms for human genomic rearrangements. *Pathogenetics* **1**, 4 (2008).